



Oviposition deterrent activity of acetone leaf extract of *Spathodea campanulata* P. Beauv. (bignoniaceae) against Chikungunya vector *Aedes aegypti* (Diptera :Culicidae)

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ABSTRACT

Mosquitoes are blood-feeding insects and serve as the most important vectors for spreading human diseases such as malaria, yellow fever, dengue fever and filariasis. The continued use of synthetic insecticides has resulted in resistance in mosquitoes. Synthetic insecticides are toxic and affect the environment by contaminating soil, water, and air then natural products may be an alternative to synthetic insecticides because they are effective, biodegradable, eco-friendly and safe to environment. Botanical origin may serve as suitable alternative bio control techniques in the future. The present study assessed the oviposition deterrent activity of acetone leaf extract of *Spathodea campanulata* against *Aedes aegypti*. For determining the influence of acetone leaf extract of *S. campanulata* on the ovipositional pattern of *Ae. aegypti*, the mosquitoes were subjected to choice – oviposition test and no – choice oviposition test. The acetone leaf extract of *S. campanulata* were found to deter mosquitoes from oviposition. Oviposition Active Index and Oviposition Deterrent Index indicated a low preference of *Aedes aegypti* to leaf extract treated medium for egg laying. From the results, it can be conclude the acetone leaf extract of *S. campanulata* was an outstanding potential for controlling the dengue vector mosquito *Aedes aegypti*.

Key words: *S.campanulata*, leaf extract, *Ae.aegypti*, oviposition deterrent activity

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INTRODUCTION

Mosquitoes are small sized insects responsible for the transmission several pathogens¹. Mosquitoes are responsible for more diseases than any other group of arthropods. Mosquito-borne diseases, such as malaria, filariasis, dengue/ DHF, yellow fever and Japanese encephalitis contribute significantly to disease burden, death, poverty and social debility in tropical countries. *Aedes aegypti*, a vector of dengue is widely distributed in the tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. *Ae. aegypti*, is also the vector of dengue hemorrhagic fever which is endemic to South East Asia, the Pacific islands, area Africa and the America². A recent estimate shows that more than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are 2 million infections, 5,00,000 cases of hemorrhagic fever and 12,000 deaths³. Indeed, the present recrudescence of these diseases is due to the higher number of breeding place in today's throwaway the public and also increasing resistance of mosquitoes to commercial insecticides. Controls of such serious diseases are becoming increasingly difficult because of the high rate of reproduction and development of resistance to insecticides in mosquitoes⁴.

Synthetic pesticides have been extensively used for mosquito control by either killing, preventing adult mosquitoes to bite human beings or by killing mosquito larvae at the breeding sites of the vector⁵. In most parts of the world, synthetic chemical larvicides continue to be applied for controlling mosquitoes but many of these chemical are toxic to human, animal and plant life and resistance can be problematic in regulating the control. There is an urgent need to develop new materials for controlling mosquitoes in an environmentally safer way, using biodegradable and target-specific insecticides against them⁶. At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is mosquito control⁷. Experience has shown that, aerial toxicants for the eradication of this mosquito are not effective, since it is highly domesticated and many adults rest indoors in hidden places such as closets. The only successful way of reducing mosquito densities to a level where dengue fever epidemics do not occur is by attacking the larval breeding places⁸. Therefore, researchers are currently exploiting natural substances to be used as insecticides for controlling larval mosquitoes.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides⁹. In this regard, India has

a rich flora that is widely distributed throughout the country. More than 2000 plants species have been known to produce chemical factors and metabolites of value in the pest control programmes¹⁰ and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes⁴. Phytochemicals are advantageous due to their eco-safety, target- specificity, non development of resistance, reduced number of applications, higher acceptability and suitability for rural areas. Plants being rich source of bioactive chemicals¹¹ and so far there is no report of resistance to plant extracts¹². Botanical insecticides also have potential uses such as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility^{13, 14}. Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: methanolic extracts of *Derris elliptica* leaves¹⁵; aqueous extract of *Senna didymobotrya* leaves¹⁶; acetone extract of *Solanum trilobatum* leaves¹¹; aqueous extract of *Gymnema sylvestre* and *Eclipta prostrate* leaves¹⁷; methanol, benzene and acetone leaf extracts of *Cassia fistula*¹⁸; petroleum ether extract of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* leaves¹⁹; aqueous and chloroform extracts of *Leucas aspera* leaf²⁰; ethanolic extract of *Datura stramonium* leaves²¹; aqueous extract of *Spathodea campanulata* leaves^{22, 23, 24}; methanolic extract of *Spathodea campanulata* leaves²⁵; petroleum ether, chloroform, and ethyl acetate extracts of *Lantana camara*, *Bauhinia racemosa* leaves²⁶; methanol extract of *Garcinia gummi- gutta* leaves²⁷; aqueous, ethanol, ethyl acetate and hexane extracts of *Gossypium hirsutum* leaf (Bt cotton)²⁸; aqueous, methanol extracts of *Veronia adoensis* leaf²⁹; ethyl acetate, methanol extracts of *Ficus krishnae* leaves³⁰; petroleum ether, benzene, ethyl acetate, chloroform :methanol (1: 1 v/v), acetone and absolute alcohol extracts of *Swietenia mahagoni* leaves³¹; methanol extract of *Annona reticulata*, *Pongamia pinnata* leaves³²; petroleum ether, N – butanol extracts of *Cassia occidentalis* leaves³³; ethanol extract of *Callistemon brachyandrus* leaves³⁴; methanol extract of *Lawsonia inermis* and *Murraya exotica* leaves³⁵; hexane, ethyl acetate and ethanol extracts of *Ricinus communis* leaves³⁶; methanol extract of *Jatropha curcas* leaves³⁷; dimethylsulfoxide extract of *Halodule pinifolia*, *Cymodocea serrulata* and *Thalasia testudinum* leaves³⁸; ethyl acetate, hexane, dichloromethane and diethyl ether extracts of *Croton sparciflorus* leaves³⁹; hexane extract of *Tragia involucrate* leaves⁴⁰; methanol extract of *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* leaves⁴¹; hexane, benzene, chloroform, ethyl acetate and methanol extracts of *Pithecellobium dulce* leaves⁴²; ethanol extract of *Morinda citrifolia* leaves⁴³; hexane, methanol, acetone, isopropanol, dimethylsulfoxide extracts of *Alstonia scholaris*, *Callistemon viminalis*, *Hyptis suaveolens*,

Malvastrum coromandelianum, *Prosopis juliflora*, *Vernonia cinerea* leaves⁴⁴; benzene, chloroform, ethyl acetate and methanol extracts of *Impatiens balsamina* leaves⁴⁵; petroleum ether, ethyl acetate, ethanol and water extracts of *Vitex negundo*, *Azadirachta indica*, *Melia dubia*, *Leucas aspera*, *Ocimum tenuiflorum*, *Eucalyptus tereticornis* leaves⁴⁶; methanol extract of *Rhizophora mucronata* leaves⁴⁷; diethyl ether, hexane, benzene and acetone extracts of *Coleus aromaticus* leaves⁴⁸; hexane, benzene, chloroform, ethyl acetate and methanol extracts of *Ageratina adenophora* leaves⁴⁹; hexane, ethyl acetate, methanol extracts of *Aegle marmelos*, *Limonia acidissima*, *Sphaeranthus indicus*, *Sphaeranthus amaranthoides* and *Chromolaena odorata* leaves⁵⁰.

It could be ascertained from the literature survey that there was no information available on the ovipositional deterrent activity of the acetone leaf extract of the *S. campanulata* against *Ae. aegypti*. Therefore, this study provides first report on the ovipositional deterrent activity of *S. campanulata* acetone leaf extract against the vector mosquito, *Ae. Aegypti* as target species.

Spathodea is a monotypic genus in the flowering plant family Bignoniaceae. It contains the single species, *Spathodea campanulata*, which is commonly known as the African Tulip Tree, Flame-of-the forest in English, Rugtoora in Hindi, Patadi in Tamil. It is a tree that grows between 7-25 m (23-82ft) tall and native to tropical Africa. This tree is planted as ornamental tree throughout the tropics and much appreciated for its very showy reddish argane (or) Crimson (rarely yellow), campanulated flowers. It is commonly planted as a street tree in south Tamil Nadu. The tree is considered evergreen but it sheds leaves in dry summers and hence it is a dry season deciduous tree. *S. campanulata* commonly employed to control epilepsy. This species has many uses in folk medicine. The flowers are employed as diuretic and anti-inflammatory while the leaves are used against kidney diseases, urethra inflammation and as an antidote against animal poisons. The leaves have furnished Spathodol, caffeic acid and other phenolic acids and flavonoids. The plant leaf is used for anti-plasmodial activity, anti-microbial activity and anti-larvicidal activity^{51, 52, 53}.

MATERIALS AND METHODS

Colonization of *Ae.aegypti*

Collection of eggs

The eggs of *Aedes aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India without exposure to any insecticide. The eggs were then brought to the laboratory and transferred to enamel trays containing water

and kept for larval hatching. They were hatched and reared and have been still maintained for many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments.

Maintenance of larvae

The larvae were reared in plastic cups. They were daily provided with commercial fish food ⁵⁴*ad libitum*. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes.

Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing water with help of a sucker. The pupae containing glass beaker were kept in side mosquito cage for adult emergence. The cage was made up of steel frame wrapped with mosquito netting. The cage had a provision (a hole) for handling of materials and animals placed in side. The hole was guarded with a sleeve which was useful to close suddenly after being used.

Blood feeding of adult *Ae.aegypti* and egg laying

The females were fed by hand every alternate day. Feeding mosquitoes on human arm for experimental purposes was suggested by^{55, 56}. Both females and males were provided with 10% glucose solution as described by⁵⁷ on cotton wicks. The cotton was always kept moist with the solution and changed every day. An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arrangement made the collection of eggs easier.

Collection of plant materials and authentication

S. campanulata P. Beauv. (Family: Bignoniaceae) leaves were collected from Government Arts college campus, Coimbatore, Southern India. The identification of the plants was authenticated at BSI (Botanical Survey of India), Coimbatore.

Preparation of plant extract

The fresh leaves of the plant *S. campanulata* were collected in our college campus area. Then the leaves brought to the laboratory. The plant leaves were observed carefully for any kind of diseases or infection and if found any, those parts were separated and not used for the experiment. The selected leaves washed with distilled water in order to clean dust or any particle stuck to them. Then the leaves kept for drying under shade at room temperature ($27 \pm 2^\circ\text{C}$) for about 2 weeks till they dried completely. The leaves were finely powdered using electric blender. 250g of leaf powder was dissolved in 200ml of acetone (as a solvent) and extracted in

the Soxhlet apparatus for 8 h over a mantle heater at 55°C. The acetone extract was concentrated using a vacuum evaporator at 45° C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in a refrigerator for later use.

Preparation of stock solution and different concentrations of leaf extract

1g of the concentrated extract of leaves of *S. campanulata* was dissolved in 100ml of acetone and kept as stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

Oviposition bioassay

Fifteen pairs of mosquitoes were kept in a cage and maintained. They were blood fed every alternate day. The effect of acetone leaf extract of *S. campanulata* on oviposition of *Ae. aegypti* was determined under two set of conditions as suggested by^{58,59,60}.

Choice oviposition test

Four egg traps containing any one of the concentrations (0.1, 0.4 and 0.8%) of the test compound and control were placed inside the cage with 15 pairs of blood fed mosquitoes. After 24 hours, the traps were taken out and the eggs present in each were separately counted. The test was replicated 10 times (10 days) for each concentration of test compound.

No-choice oviposition test

Egg trap containing any one of the test concentrations of the test compound was placed at the time in the cage with 15 pairs of mosquitoes, along with a control trap. After 24 hours, the containers were removed and the number of eggs was counted. The trial was repeated 3 times for each concentration.

Oviposition Active Index (OAI) was calculated as detailed by⁶¹ using the formula,

$$\text{OAI} = \frac{N_t - NS}{N_t + NS}$$

Where

N_t is the total number of eggs in test solutions and

NS is the total number of eggs in control

This would indicate whether the effect of the compound on oviposition is positive or negative.

Further, the percentage of oviposition deterrence (oviposition deterrent index of Lundgren, 1975) was determined according to the formula given by⁶².

$$\text{Oviposition Deterrent Index (ODI)} = \frac{B-A}{A+B} \times 100$$

Where

A – is the number of eggs laid on treated

B – is the number of eggs laid on control.

The data were statistically examined using Student's *t*- test.

RESULTS AND DISCUSSION

Effect of acetone leaf extract of *S. campanulata* on oviposition of *Ae.aegypti*

For determining the influence of acetone leaf extract of *S. campanulata* on the ovipositional pattern of *Ae.aegypti*, the mosquitoes were subjected to choice – oviposition test and no – choice oviposition test. The data were substituted under appropriate formulae to calculate oviposition active index (OAI) and oviposition deterrent index (ODI). The results are furnished tables 1&2.

Table 1. Changes in the indices of oviposition deterrence at different concentrations of acetone leaf extract of *Spathodea campanulata* against *Aedes aegypti* under choice oviposition test.

Parameters	Control	Concentrations (%)		
		0.1	0.4	0.8
Total number of eggs laid*	721	515	195	52
Percent oviposition	48.61	34.72 [#]	13.14 [#]	3.506 [#]
Percent reduction in oviposition over control		71.42	27.04	7.212
Reduction in number of eggs compared to control		206	526	699
Oviposition active index (OAI)		-0.166	-0.574	-0.865
Oviposition deterrent index (ODI)		16.66	57.42	86.54

*, Total number of eggs in 10 replicates.

[#], Significantly different from control (P<0.001).

Table 2. Changes in the indices of oviposition deterrence at different concentrations of acetone leaf extract of *Spathodea campanulata* against *Aedes aegypti* under no – choice oviposition test.

Parameters	Concentrations (%)					
	C	0.1	C	0.4	C	0.8
Total number of eggs laid*	327	237	261	126	451	55
Percent oviposition	57.97	42.02 [#]	67.44	32.55 [#]	89.13	10.86 [#]
Percent reduction in oviposition over control		72.47		48.27		12.19
Reduction in number of eggs compared to control		90		135		396
Oviposition active index (OAI)		-0.159		-0.348		-0.782
Oviposition deterrent index (ODI)		15.95		34.88		78.26

*, Total number of eggs in 3 replicates.

[#], Significantly different from control (P<0.001).

Choice oviposition test

Mosquitoes showed more preference towards control ovitrap for oviposition, though; media of different concentrations of acetone leaf extract of *S. campanulata* were available along with control (Choice oviposition test). The total number of eggs laid in ovitraps containing any

concentration of the acetone leaf extract of *S. campanulata* was always less than that in the control. Among the total number of eggs laid, 48.61 % was present in control medium when placed along with ovitraps with 0.1, 0.4 and 0.8 % acetone leaf extract of *S. campanulata* in which appeared 34.72, 13.14 and 3.506 % of eggs respectively. This was also indicated by ODI values (16.66, 57.42 and 86.54). Rate of oviposition in ovitraps with any concentration of test compounds was significantly ($P < 0.001$) less than in control.

No - choice oviposition test

The ovipositional deterrence of acetone leaf extract of *S. campanulata* against *Ae. aegypti* was also confirmed by the results of 'no - choice test' where ovitrap with any one of the concentrations accompanied the control. Percent oviposition in 0.1, 0.4 and 0.8 % of acetone leaf extract of *S. campanulata* was 42.02, 32.55 and 10.86 % which were significantly ($P < 0.001$) less compared to their control counterparts 57.97, 67.44 and 89.13 %, respectively. The data of no - choice oviposition test clearly exhibited interference of acetone leaf extract of *S. campanulata* on the oviposition preference of mosquitoes.

Oviposition active index for both ethanolic leaf extract of *Ocimum kilimandscharicum* (OK) and *Ocimum suave* (OS) experiments egg lay in a negative side ranged from -1% to -0.19%. It was showed that OS and OK deter oviposition in *An. gambiae*⁶³; ethanolic extract of *Pongamia pinnata*, *Coleus forskohlii* and *Datura stramonium* leaves reduced egg laying by 97.62%, 77.3%, 100% against *Ae. aegypti* and 59.10%, 39.22%, 82% against *Cx. quinquefasciatus* at higher concentration (0.1%)⁶⁴; the ethanolic leaf extract of *Sloanea trilobatum* was tested under laboratory conditions for oviposition deterrent activities against the adult *An. stephensi* and the concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1% reduced egg laying by females from 18 to 99%⁶⁵; 100% oviposition deterrence was obtained with *Melia azedarach* leaf extract at 1g/L against *Ae. aegypti*⁶⁶; the oviposition active index (OAI)⁶⁶ value of acetone, ethyl acetate, and methanol extracts of *Aegle marmelos*, *Andrographis lineate* and *Cocculus hirsutus* leaf at 500 ppm against *An. subpictus* were -0.86, -0.87, -0.90, -0.78 and -0.87, -0.86, -0.91, -0.94 and -0.86 respectively and the OAI values revealed that the solvent plant extracts have deterrent effect, and they caused a remarkable negative response resulting an oviposition of very few eggs⁶⁷; ethanolic leaf extract of *Andrographis paniculata* observed against *An. stephensi* and OAI values for the species were -0.28, -0.45, -0.49 and -0.59 for extract concentrations of 29, 35, 41 and 46 ppm respectively⁶⁸; in oviposition deterrent activity, the highest concentration of (0.1%) ethanolic leaf extract of *Vitex negundo* produce 94.2% in *Ae. aegypti*, 96.4% in *An. stephensi* and 99.8% in *Cx. quinquefasciatus*⁶⁹; the highest concentration of (0.1%) acetone, chloroform, hexane,

petroleum ether and ethanol extracts of *Annona squamosa* leaves produce oviposition deterrent activity 99.6% against *An. stephensi*, 92.4% against *Cx. quinquefasciatus* and 92.4% against *Ae. aegypti* respectively⁷⁰; acetone extract of *Prosopis juliflora* leaf found to be strong oviposition deterrent which inhibited egg laying (OAI-0.466) at 100 ppm.⁴⁴

The oviposition is one of the most important events in the life cycle of mosquitoes⁷¹. If oviposition is prevented the mosquito life cycle is disrupted and population growth is reduced. The present oviposition study shows that the acetone leaf extract of *S. campanulata* act as oviposition deterrent, this indicates that *Ae. aegypti* mosquitoes were acutely sensitive to phytochemical stimuli and respond to the odor of the leaf extract. The strong odor produced by higher concentration of acetone leaf extract produce maximum effective repellence against oviposition. The mosquitoes are known to select or reject their specific oviposition sites by sensing chemical signals that are detected by sensory receptors on the antenna⁷².

CONCLUSION

From the results, it can be concluded that ovipositional deterrence activity against dengue vector mosquito *Aedes aegypti* is due to phytochemicals present in the acetone leaf extract of *Spathodea campanulata*. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other plants. *Spathodea campanulata* acetone leaf extracts may contribute greatly to save environment and to an overall reduction in the population density of dengue vector *Aedes aegypti*.

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